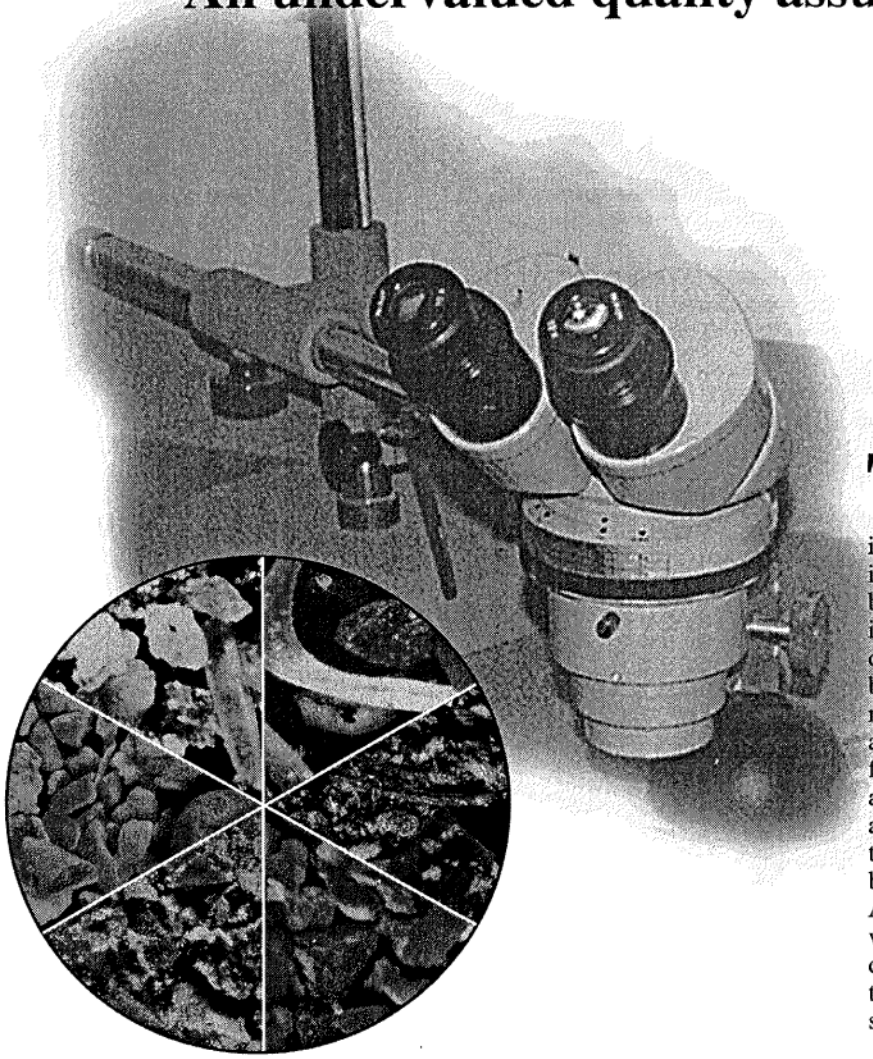
EXHIBIT R

Feed microscopy

An undervalued quality assurance technique



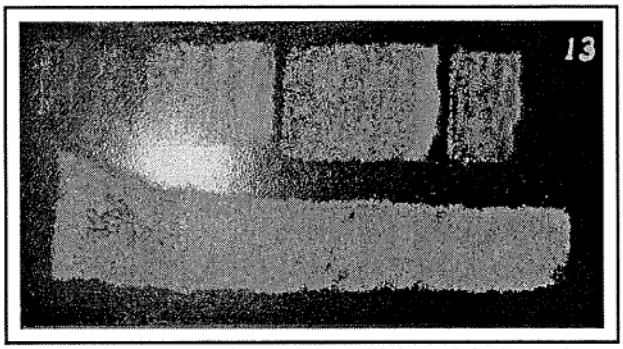
The American Association of Feed Microscopists (AAFM) was chartered in 1953, in Lexington, Kentucky, by a group of 30 individuals. These founding members (including Lewis Barefield, who is still active) were state feed-control officials, state chemists, and members of industry who recognized the need for rapid, inexpensive, and reliable methodology that could ensure feed quality and labeling compliance. After 45 years of independent annual meetings and short courses, the AAFM sought to expand its visibility by becoming a division of AOCS. In September 1997, AAFM was officially recognized as the tenth division of AOCS, and is known as the Feed Microscopy (AAFM) Division.

The American Association of Feed Microscopists (AAFM) became an AOCS division in 1997. Just as AOCS had its origins among cottonseed analysts seeking to improve commercial analytical techniques, the AAFM was begun by feed analysts looking for rapid, simple analytical methods. This article provides background information on feed microscopy and how it is used. The article was written by former AAFM President James V. Makowski, a faculty member in the Department of Natural Science at Messiah College in Grantham, Pennsylvania, and owner of Windsor & Associates Laboratory, 894 Hawthorn Ave., Mechanicsburg, PA 17055.

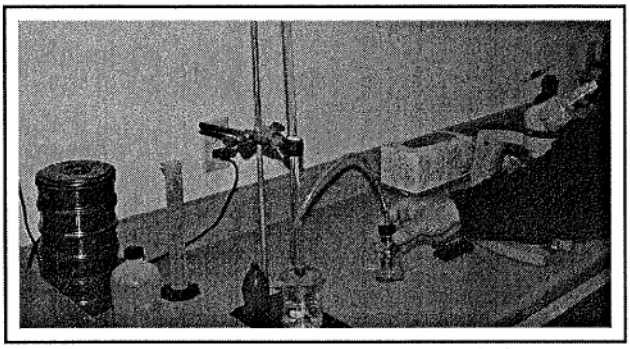
What is feed microscopy?

Feed microscopy is the art and science of identifying feed ingredients and minerals in animal feeds and, more recently, in human foods, using stereo and compound microscopy. The goal of feed microscopy is to provide manufacturers and consumers a technique for determining which feed ingredients and minerals are present in a given feed, and that the feed or food is nutritious and safe for animal maintenance, production, or human consumption.

Perhaps the greatest value of feed



Computerized observation board containing original sample (bottom) and sample fractions arranged from highest/greatest to lowest/least density (top, left to right)



Buret used to trap and measure carbon dioxide

microscopy is that it is a versatile technology for rapid, simple, quality control. It is an ideal complement to chemical and chromatographic analyses. Chemical analyses determine the protein, fat, and fiber content of a feed; microscopy identifies the ingredients, as well as providing precise information about the nutritional value and quality of a feed. Microscopy is particularly well suited for identifying and quantifying contaminants and adulterants *via* their physical characteristics, when no rapid alternative chemical or physical tests are available. Additionally, microscopy requires minimal sample preparation and lead time. Because the concepts are simple and the equipment is relatively inexpensive, feed microscopy complements chemical analyses and should be an essential part of every quality assurance program.

How do we use feed microscopy?

The feed microscopist can provide data which are not usually gained by chemical analyses. Some of the most effective applications are:

- Identifying ingredients,
- Evaluating finished feeds,
- Determining the presence of extraneous materials (weed seeds, etc.),
- Evaluating quality and processing, and
- Recognizing contamination from insects or infestation by fungi.

Many feed mills use an "eyeball" approach to visual inspection of raw ingredients and finished products. Although visual observation is subjective, no single chemical screening can detect and identify all the possible contaminants and adulterants that can be present in an ingredient or finished product. For example, one feed mill unknowingly substituted hexane-extracted dehulled lupin bean meal for soybean meal. The lupin meal, which was used in several swine rations on farms in North Dakota, resulted in decreased performance, increased incidence of stillborn pigs, reduced litter size, and death losses. Thus, the ability to identify specific feed ingredients—even with a simple hand lens—can be an asset to a mill's quality assurance program.

Another major use of feed microscopy is to evaluate finished products. Testing is reasonably easy for both granular and pelleted feeds. A feed microscopist can quantify within ±2–3% the amounts of various major and some obvious minor ingredients in blended and pelleted products. Chemical flotation/separation procedures using chloroform and other solvents allow the feed microscopist to separate ingredients into density-dependent fractions. Such techniques permit the microscopist to identify and to quantify major ingredients and minerals in finished products.

The identification of contaminants and adulterants in feed ingredients and finished feeds is probably the

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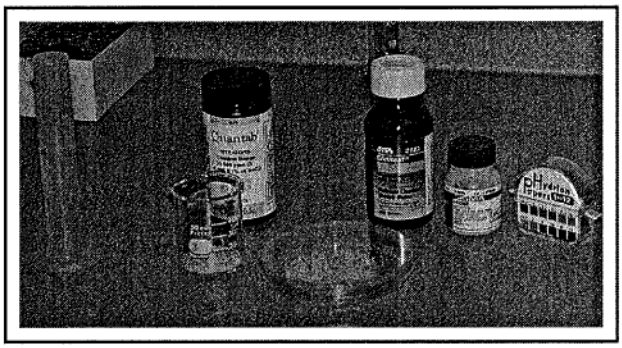

Materials used to ascertain chlorides, sugar, urea, and pH

Weighing sample fraction, prior to transfer to observation board

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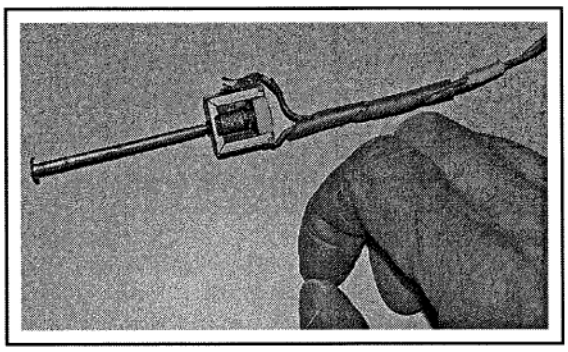

Simple, homemade laboratory stirrer

most important application of feed microscopy in a routine quality assurance program. Adulterated ingredients may be substituted by suppliers in an effort to cut costs and increase profit. A trained feed microscopist can identify and quantify the presence of contaminants and adulterants in both raw ingredients and finished products. Contaminants, such as gossypol (in cottonseed meal) or poisonous weed seeds, can be lethal if ingested in high enough quantities. In the current U.S. legal environment where everyone is a potential target for a lawsuit, feed manufacturers and ingredient suppliers must utilize every technique to ensure that the finished product is of expected quality.

One important aspect of feed microscopy is the evaluation of ingredient quality. Specified ingredients in the proper amounts may be present in a feed, but if the ingredients are of poor quality, or if they have been improperly processed, the resulting feeds will have diminished nutritional value. For example, feather meal, if not hydrolyzed, is indigestible to monogastrics and ruminants. Overcooking of some ingredients, such as soybean meal, renders them less digestible. Damages from the high temperatures of feed ingredient driers or from weathering in the field also may lower feed ingredient quality. Quality assessments with respect to these conditions can be made by an experienced microscopist.

The possibility of contamination by toxins from weed seeds and certain insects can be indicated by the presence of even tiny fragments of the suspect organisms. Similarly,

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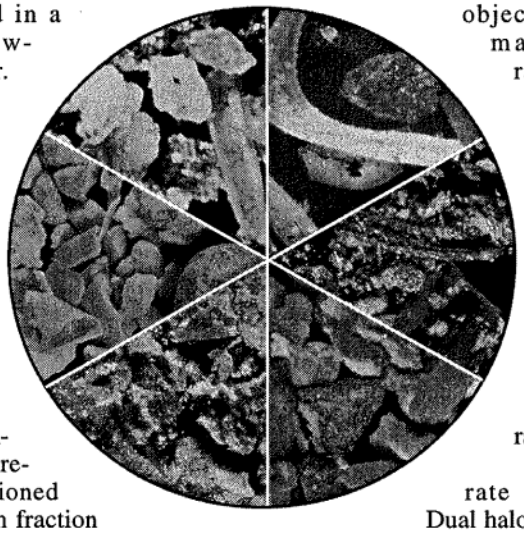
microscopic identification of certain molds and mold spores assists in the determination of mold metabolites and feed-refusal factors that may have not been well defined chemically or were missed by routine mycotoxin screening. The presence of insects or mold in feeds may indicate improper processing or storage and should be detected quickly by the feed microscopist. Improperly stored feed also may be susceptible to bacterial growth, and ingestion of bacterial toxins can lead to many animal disorders or death. The presence of grain mites in a feed may cause animals, particularly poultry, to refuse a feed. Although these insects are not toxic or pathogenic, they have spines which may decrease intake due to oral and gullet irritation.

Types of feed microscopy

Feed microscopy may be divided into two major types—qualitative and quantitative. Qualitative feed microscopy is the identification and evaluation of ingredients and foreign material *via* surface features and/or cellular characteristics. Qualitative methods are important, not only for quality assurance but also for regulatory protection, to ensure that the ingredients listed on the label are indeed present in the sample. Once a feed mill has established a level of acceptable quality, qualitative evaluations are relatively simple. Particular attention is given to the smallest particles, because adulterants are usually finely ground to escape detection. Larger particles—over 10 mesh—are also examined for the presence of fibrous contaminants.

Quantitative microscopy is the subjective, proportioned measurement of ingredients in finished feeds, or of contaminants and adulterants in ingredients. Some methods for quantifying mixtures include handpicking and weighing individual ingredients, comparing ingredient ratios to known ratio standards, and counting cells. In my laboratory, mixed ingredients are ground in a ripple-plated hand grinder to break them down without destroying cellular integrity. The ground portion is then subjected to a flotation/separation procedure using two solvents of different densities, such as chloroform and petroleum ether. This procedure typically produces from four to six density-dependent fractions that are arranged on an examination board in a high- to low-density order. Because feed ingredients have different densities, they separate into more homogenous fractions than samples examined by the previously mentioned methods. Each fraction is weighed on an analytical balance prior to being placed on the examination board. Fractions are then examined under a stereomicroscope, and the percentages of ingredients in each fraction are estimated and then combined to produce a total of each ingredient present in the sample. This procedure can often produce results that have a ±3–4% accuracy for high-quantity ingredients and ±0.5–1% for ingredients present in lower quantities.



The microscopist can verify the results of quantification if the protein level has been determined for the sample. Once the percentage of each ingredient is estimated, the microscopist can multiply each protein-containing ingredient by an average tabular value protein. If the calculated sum total of protein from each of the ingredients closely approximates the feed-label guarantee, then the ingredient percentages derived by the microscopist should be close to the actual crude protein.

Tools of the microscopist

The minimal basic equipment for the feed microscopist should consist of the following:

• Samples of known ingredients and ingredient mixtures for quantifying and comparison. This sample "library" should contain as many different ingredients and problem samples as possible.

• A stereomicroscope with wide-field eye pieces, objectives, and a magnification range of 10–30X. Parallel optics are preferable to convergent systems in order to reduce eye strain. A zoom objective is preferred for rapid work.

• A separate illuminator. Dual halogen or halogen fiber-optic light pipes are preferable for cool, white light, but a low-cost halogen desk lamp is an acceptable alternative.

• A compound microscope for fine particle analyses, and cellular and subcellular confirmations. It should have a binocular head with 10X plan achromatic (flat-field) oculars coupled with 4X, 10X, 40X and 100X plan achromatic objectives. A mechanical stage and adjustable condenser and diaphragm are important for proper contrast adjustments. A polarizing element and a rotating stage enable the microscopist to examine birefringent, crystalline structures.

• A balance, mechanical or electronic, capable of 0.01 g accuracy. Balances allow precise weighing of flotation/separation fractions, increasing the accuracy of reported findings.

• Evaporating dishes, a method for removing heavy solvent vapors, and simple laboratory stirrers allow flotation/separation procedures to be carried out. Other handheld instruments, such as stainless steel forceps, dissecting needles and a stainless steel micro spatula, aid in sample handling and manipulation of individual particles while using the stereomicroscope. Some microscopists keep a small

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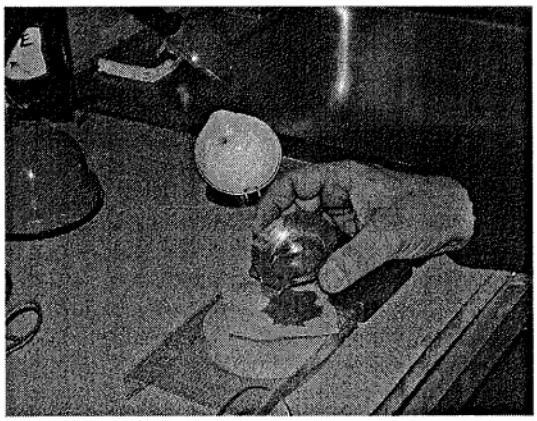
brush, a scalpel (or single-edged razor blade), and spot plates at their work station. Small containers, such as petri dishes, small beakers, disposable aluminum pans, and plastic or paper cups are also helpful.

A feed microscopy laboratory can be set up in a small office or along the side of an existing quality assurance laboratory. The equipment is relatively inexpensive for the information it can help generate within a short period of time. The most expensive component is the training of the feed microscopist, which can be obtained through various short courses and seminars. These options allow for the hands-on examination of feed products necessary to recognize feed ingredients. Because of the vast range of ingredients present in feed products, learning this material from textbooks is difficult. The Feed Microscopy (AAFM) Division of the American Oil Chemists' Society offers both basic and advanced short courses in conjunction with their annual meeting.



Pouring samples fraction onto collection media

As feed microscopy becomes more widely known, it is being recognized as a valuable technology that is ideally suited to complement the chemical methodology used in quality control laboratories. Feed microscopy offers rapid, reliable analysis when more objective methods are not available. Feed microscopy offers some unique attributes that decrease the probability of feed and food problems. However, the methodologies must be applied correctly and the data must be interpreted correctly to yield the maximum benefits for each quality-control program. ■

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